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Fortification of milk with phytosterol and its effect on sensory and physicochemical properties

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Abstract

Phytosterols are a group of lipophilic steroid alcohols found in plants, which have been shown to lower cholesterol when supplemented in the diet. A commercial phytosterol preparation was added to milk in the form of an oil-in-water emulsion. For the preparation of an emulsion, diacetyl tartaric acid ester of mono- and diglycerides was used as an emulsifier and butteroil was used as a source of fat. Three emulsion formulations, i.e. A (8% phytosterols), B (10% phytosterols) and C (12% phytosterols), were prepared in which the levels of emulsifier (6.5%) and butteroil (10%) were kept constant, and each emulsion was added to milk at a rate of 5% (w/w). Based on sensory evaluation, B-emulsion formulation was selected for fortification of milk. The phytosterol content of the fortified milk determined by reverse-phase high-performance liquid chromatography was 410.8 mg/100 g. No significant loss in the initial content of phytosterol was observed after 1 week of storage. Sensory and physicochemical analyses indicated that significant differences were not observed between control and fortified milk samples up to 7 days of refrigerated storage. The present study suggests that it is feasible to add phytosterol as a functional ingredient in milk in the form of water-soluble emulsion to enhance health benefits of consumers. Two servings of such fortified milk per day provide almost the entire recommended daily requirement of phytosterol.

Keywords

oil-in-water emulsion • physicochemical properties • phytosterol-enriched milk • sensory properties

Introduction

Currently, great efforts are focussed on reducing the risk of coronary heart disease through dietary intervention. A number of dietary agents, including plant sterols (stanols), were found to interfere with cholesterol absorption and to lower its level in serum (Kamal-Eldin and Moazzami, 2009). Phytosterols or plant sterols are found in seeds, vegetable oils and cereals with a molecular structure very similar to that of cholesterol. The most frequently found phytosterols in nature are β-sitosterol, campesterol and stigmasterol (Lengyel *et al.*, 2012). These molecules are able to displace cholesterol during micelle formation in the intestine due to their higher hydrophobicity, thus reducing cholesterol absorption (Calpe-Berdiel *et al.*, 2009). Plant sterols might also protect against certain types of cancer such as stomach, colon, breast and prostate (Woyengo *et al.*, 2009). Although phytosterols are widely found in diets rich in plant matter, the amounts of phytosterols ingested from a normal diet range from 150 to 440 mg/day (García-Llatas and Rodríguez-Estrada, 2011), which is barely adequate for obtaining a significant health benefit. For this reason, phytosterols have become an interesting ingredient for use as food supplements and in food formulation.

Recently, the use of health claims for foods containing phytosterols was revised by the US Food and Drug Administration (FDA, 2010), and it reported that a daily dietary intake of 2 g of phytosterols is required to demonstrate relationship between phytosterol consumption and lowering of cholesterol for reduced cardiovascular disease risk. According to epidemiological and clinical studies, the daily intake of 2 g of phytosterols could result in a mean reduction of 8.8% of low-density lipoprotein cholesterol (Demonty *et al.*, 2009). Based on these studies, several functional food formulations have been developed in order to exploit the health benefits of phytosterols; some of these formulations include dairy products, snack bars, sausages, bakery products, spreads, cereals, salad dressings, breads, orange juice and chocolate (García-Llatas and Rodríguez-Estrada, 2011; Gonzalez-Larena *et al.*, 2011) at doses that range from 2 to 3 g (Kmiecik *et al.*, 2011).

The application of phytosterols in food formulation is rather challenging as they are poorly soluble in the aqueous medium and typically have a melting point range of 120–140°C. Although phytosterols are soluble in fat and oil, the amount of phytosterols

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soluble in the fat and oil phase is very small (Leong *et al.*, 2011). For the sake of product formulation and increasing the rate of use, phytosterols need to be converted into the form of a suitable food additive through some processing or formulation conversion (Wu *et al.*, 2007). An emulsion system may be a simple and better approach. Phytosterols made into a water-soluble form by emulsification can be applied in a wide range of food products. Some clinical trial results indicate that sterol-enriched milk and milk-based fruit beverages are effective at reducing concentrations of serum cholesterol (Noakes *et al.*, 2005; Gonçalves *et al.*, 2007; Garcia-Llatas *et al.*, 2015). However, no information is available related to physicochemical and sensory properties of phytosterol-enriched milk.

In the present study, attempts were made to prepare an emulsion containing phytosterols for the easy incorporation of phytosterols in milk. The physicochemical and sensory characteristics and the level of phytosterols in the fortified milk were analysed and compared with those of the control milk containing no phytosterol during storage at 4°C for 1 week.

Materials and methods

Materials

Raw bovine milk was obtained from the Livestock Research Centre of the National Dairy Research Institute (Bengaluru, India). The raw milk was warmed to 40°C and separated into cream and skim milk using a cream separator (Kamdhenu, New Delhi, India). The skim milk (<0.5% milk fat) and cream (~40% fat) were appropriately blended to prepare milk with 3% fat and 8.5% non-fat solids. A commercial food-grade phytosterol preparation was obtained from Bio-gen Extracts Pvt. Ltd., Bengaluru, India. According to the manufacturer, it contained 42.38% of β-sitosterol, 24.81% of stigmasterol, 26.73% of campesterol and 1.84% of brassicasterol. Diacetyl tartaric acid esters of mono- and diglycerides (DATEM; Panodan® 165 Kosher) were obtained from Danisco India Pvt. Ltd., Gurgaon, India.

Chemicals and standards

High-performance liquid chromatography (HPLC)-grade methanol, water and hexane were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Reference standards of β-sitosterol, stigmasterol and campesterol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Standard cholesterol was procured from Qualigens Fine Chemicals (Mumbai, India). All other chemicals used in the study were of analytical reagent (AR) grade and were procured from Qualigens Fine Chemicals (Mumbai, India).

Preparation of butteroil

The cream collected was kept at 7–8°C for 10–12 h and then churned into butter using a domestic mixer (National, Bengaluru, India). The butter was washed with cold water to remove excess buttermilk and was heated with continuous stirring in a stainless steel container. The heating was adjusted so that the temperature rose very slowly and did not exceed 100°C. Finally, the clarified fat was filtered using Whatman Grade No. 4 filter paper to obtain butteroil with a moisture content of <0.3%.

Preparation of oil-in-water (o/w) emulsion

DATEM were used as an emulsifier and butteroil was used as a source of fat for dissolving and dispersing phytosterols. The suspension of butteroil, emulsifier and phytosterols was heated at 120°C in a paraffin oil bath and mixed at 700 rpm for 2 min using a laboratory blender (REMI, Mumbai, India) with hot distilled water (90°C) added to the above suspension. Mixing was continued for 5 min at 1,500 rpm to achieve a stable emulsion.

A formulation was prepared by mixing the four ingredients (emulsifier, phytosterols, butteroil and water). Three combinations (A, B and C) of o/w emulsion were prepared according to formulations given in Table 1. The levels of emulsifier (6.5 g) and milk fat (10 g) were kept constant in all the three combinations.

Preparation of phytosterol-enriched milk

The prepared phytosterol emulsion was added to milk (3% fat; 8.5% non-fat solids) at a rate of 5% (w/w). The milk and added emulsion (A, B or C) were mixed, homogenised at 60°C (17.24 and 3.45 MPa) (H-102; GOMA, Mumbai, India), heated to 75°C/15 s and cooled to 4°C. Emulsion was not added to control milk but was treated similarly. The milk samples were stored at 4°C for 1 week and evaluated for sensory and physicochemical characteristics and emulsion stability on 0, 2, 4 and 7 days of storage.

Sensory evaluation

A trained sensory panel assessed the coded milk samples at random, according to the methodology described by Watts *et al.* (1989). Sensory evaluation of fortified milk samples was carried out with a 25-member panel (ages 22–45 yr) consisting of scientists, students and technical staff of the National Dairy Research Institute (Bengaluru, India) with previous knowledge on sensory evaluation of dairy and dairy-associated products. Samples were placed in polypropylene containers and conditioned at room temperature for 15 min before testing. They were analysed for sensory properties such as colour, consistency, flavour and overall acceptability. Each panellist was asked to taste the milk samples and rate the sensory parameters on 9-point hedonic scale. The scores were from

Table 1. Combinations of emulsion formulations containing phytosterol (w/w)

Combinations	Phytosterol (g)	Butteroil (g)	Emulsifier (DATEM) (g)	Water (g)
A	8	10	6.5	75.5
B	10	10	6.5	73.5
C	12	10	6.5	71.5

DATEM= diacetyl tartaric acid esters of mono- and diglycerides.

9 (like extremely) to 1 (dislike extremely). Water was provided to rinse the palate before and after tasting the sample. The test was conducted three times. Mean values from control and fortified samples for each of the three replications by each panellist were considered before statistical analysis.

Physicochemical analysis

The pH of the milk samples was determined using a pH metre (CyberScan 2500; Eutech Instruments, Mumbai, India). Titratable acidity was determined by the method described in AOAC (2005). The fat and total solids contents of fortified phytosterols and control samples were determined by the gravimetric method as described in AOAC (2005). A Brookfield viscometer (Brookfield LVDV-II+Pro; Brookfield Engineering Labs., Inc., Middleboro, MA, USA) with jacketed small sample adapter and S-18 spindle was used to measure viscosity. A total of 5 mL of sample was added to the sample cup and allowed to stand for 60 s before measurements were taken, and the temperature of the sample was maintained at 30°C. Readings were taken at 100 rpm, and results were expressed in centipoises (cP).

The value of thiobarbituric acid (TBA) was used to assess lipid peroxidation of milk samples as described by King (1962). The TBA value expressed as malondialdehyde (MDA) (mm/kg) was calculated using a molar extinction coefficient of 1.56×10^5/M cm.

Emulsion stability

The emulsion stability of fortified milk samples was determined over 7 days of storage following the procedure of Wehr and Frank (2004). Total lipid content (%) in the top and bottom layers of phytosterol-fortified milk was determined by the gravimetric method using a Mojonnier extraction flask (AOAC, 2005). Each sample was poured into a 100 mL graduated cylinder, capped and stored at 4°C until the day of analysis. On the day of analysis, the top and bottom layers (10 g) were collected using 10 mL pipettes. The total lipid content of the top and bottom layers were compared to one another to evaluate the emulsion stability of the fortified milk.

Phytosterol analysis

The phytosterols were extracted by direct saponification of the samples with slight modification of the method given by Fletouris *et al.* (1998). Each milk sample was accurately weighed (0.2 g) into a sample preparation tube to which 5 mL of 0.5 M methanolic KOH solution was added. The tube was capped tightly, and its contents were vortexed for 15 s. The lower half of the tube was then immersed in a water bath maintained at 80°C for 15 min, removing the tube every 5 min to vortex for 10 s. Following heating, the tube was cooled under running tap water, 1 mL of water and 5 mL of hexane were added and the contents were vortexed vigorously for 1 min and then centrifuged for 3 min at 2,500 rpm (280 × *g*). An aliquot of the upper phase was transferred to a 50 mL conical flask and evaporated to dryness under nitrogen. The residue was dissolved in 10 mL of methanol and filtered through a 0.22-mm membrane filter (polyvinylidene difluoride; HiMedia, Mumbai, India), and the filtrate was used for HPLC analysis.

Once the sterols were isolated from milk, a method based on reversed-phase (RP)-HPLC separation combined with ultraviolet (UV) detection was used for phytosterol analysis. The samples were analysed for phytosterol levels by RP-HPLC with the Waters 515 Solvent Delivery System, a 20 mL injection loop (Rheodine, P^{IN7725}) and a Waters 2489 spectrophotometric UV/visible detector at 205 nm. Chromatographic separation was carried out using a SunFire™ C_{18} column (4.6 × 250 mm, 5 mm) (Waters, Milford, MA, USA). Isocratic elution was performed with the mobile phase of methanol and water (99:1) at a flow rate of 1.0 mL/min (Holen, 1985). An isocratic elution was chosen since it is simple, requires only one pump and minimises the variation of baseline and ghost peaks. The column temperature was set at 30°C, and the injection volume was 20 mL. Reference standards were used to determine the retention time of the phytosterols and cholesterol. Data were evaluated by the software Empower Build 2154 (Waters, Milford, MA, USA).

Statistical analysis

The entire experiment was replicated three times, and mean and standard deviation (s.d.) values were calculated. All statistical analyses were performed using SYSTAT 6.0.1 software, and statistical significance was set at $P < 0.05$. Analysis of variance was used to determine differences among treatment mean values using the *post hoc* test (Bonferroni adjustment).

Results and discussion

Sensory evaluation

There was no significant difference in scores for both colour and consistency between the control sample and milk with added emulsion, at a level of 5%, containing different levels of phytosterols (Table 2). The flavour scores of milk added with emulsion containing different levels of phytosterols (A, B or C) decreased when compared to those of the control sample. The degree of flavour preference decreased with an increase in the concentration of phytosterols in the emulsion used. However, scores for flavour of milk added with A and B formulations of phytosterols did not differ significantly compared to those of the control sample, while the score of milk added with C formulation differed significantly. For overall acceptability, milk added with A or B formulations appeared to be equally preferred as the control sample, while the one added with the C formulation differed significantly ($P > 0.05$). Raju *et al.* (2013) reported that the sensory scores of phytosterol-enriched, low-fat flavoured milk (at 2, 2.5 and 3%) decreased with the increased level of phytosterols. Izadi *et al.* (2015) developed enriched yoghurt with phytosterol dispersions, and sensory results indicated that there was no significant difference between the enriched yoghurt and control sample on texture, appearance, flavour and overall acceptance. In the present study, milk added with emulsions A or B was comparable to control milk in all the sensory parameters. Since the B formulation contained a higher level of phytosterols, this emulsion was selected for further trials.

Physicochemical analysis

The phytosterol-enriched milk (B) showed a significant decrease in pH and a numerical increase in acidity compared to the control sample (Table 3). Phytosterol-enriched milk had a higher viscosity value and differed significantly from the control sample. The rise of viscosity in the fortified milk might be due to the addition of emulsion. In addition, the viscosity of milk beverages increases as fat content increases (Phillips *et al.*, 1995). Therefore, the added fat from the emulsion might also have had an effect on the viscosity of the fortified milk. The viscosity of the fortified milk, however, was within the range generally observed for milk.

The fat content of phytosterol-enriched milk increased significantly compared' to that of the control sample. The fat content increased by nearly 1% in the enriched milk samples. Similarly, the total solids content also increased significantly in the phytosterol-added milk compared to the control sample as expected. The levels of fat and total solids in the fortified milk were, however, within the range normally found in cow milk.

Phytosterol analysis

Of the four sterols tested, cholesterol eluted first, followed by stigmasterol, campesterol and β-sitosterol; the last one had the highest molecular weight and two double bonds, hence eluted last (Figure 1). The entire chromatographic run was for 25 min and sterols were eluted between 17 and 23 min. On the C_{18} column, stigmasterol and campesterol eluted as a single peak. The other sterols like cholesterol and β-sitosterol were baseline separated (Figure 1f). Changes in HPLC conditions like column temperature, flow rate, different mobile phases and their proportions could not separate stigmasterol and campesterol.

Table 2. Sensory scores of milk added with emulsion containing different levels of phytosterols

Sensory attributes	Control	A	B	C
Colour	8.27 ± 0.16[a]	8.21 ± 0.21[a]	8.2 ± 0.2[a]	8.17 ± 0.22[a]
Consistency	8.19 ± 0.23[a]	8.19 ± 0.17[a]	8.16 ± 0.23[a]	8.16 ± 0.23[a]
Flavour	8.19 ± 0.16[a]	7.93 ± 0.17[a]	7.76 ± 0.13[a]	7.18 ± 0.12[b]
Overall acceptability	8.17 ± 0.18[a]	7.98 ± 0.19[a]	7.77 ± 0.13[a]	7.27 ± 0.05[b]

Data represent mean ± s.d. For each attribute, mean values with different superscripts (a, b) differ significantly ($P < 0.05$) from each other (by *post hoc* analysis). A, B and C are phytosterol emulsion formulations. s.d. = standard deviation.

Table 3. Physicochemical properties of phytosterol-enriched milk

Parameters	Control	Phytosterol-enriched milk*
pH	6.67 ± 0.02[a]	6.57 ± 0.01[b]
Acidity (% of lactic acid)	0.15 ± 0.005[a]	0.17 ± 0.005[a]
Viscosity (cP)	1.59 ± 0.03[a]	1.89 ± 0.07[b]
Fat (%)	2.99 ± 0.02[a]	4.04 ± 0.07[b]
Total solids (%)	11.49 ± 0.05[a]	12.53 ± 0.05[b]

Data represent mean ± s.d. For each parameter, mean values with different superscripts (a, b) differ significantly ($P < 0.05$) from each other. *Fortified milk containing B emulsion formulation. cP = centipoises; s.d. = standard deviation.

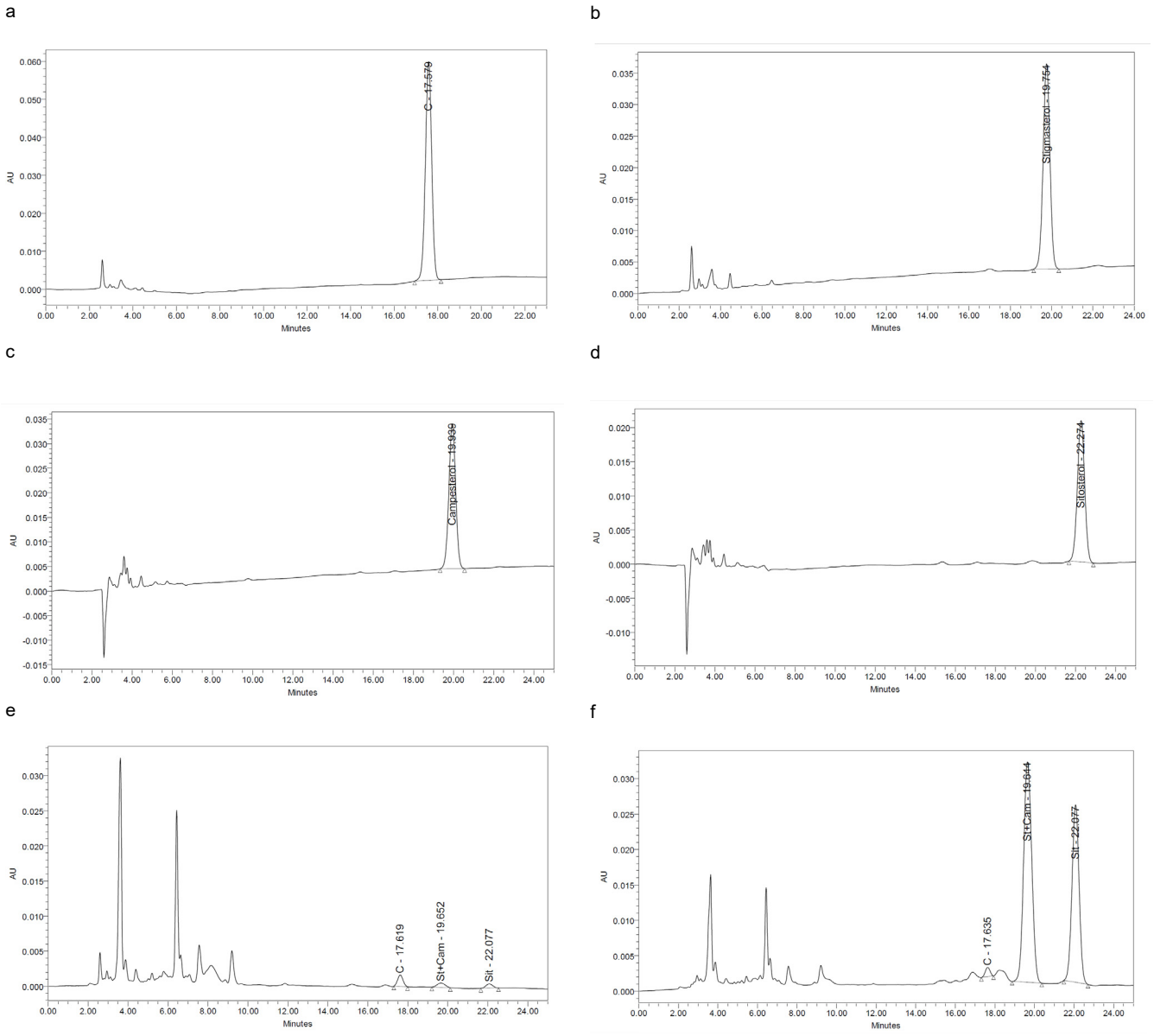

Figure 1. RP-HPLC chromatogram of sterols in standards and milk samples. (a) Cholesterol, (b) stigmasterol, (c) campesterol, (d) β-sitosterol, (e) control milk and (f) fortified milk. RP-HPLC = reversed-phase high-performance liquid chromatography; AU = absorbance units; C = cholesterol; St+Cam = stigmasterol and campesterol; Sit = β-sitosterol.

The individual and total phytosterol contents of the control milk and fortified milk are shown in Table 4. Figure 1e and f represent typical chromatograms of control and fortified samples. The cholesterol and total phytosterol content of the control sample were 10.01 and 0.30 mg/100 g, respectively. Piironen *et al.* (2002) reported that the cholesterol contents varied between 5.6 and 6.4 mg/100 g in semi-fat (1.5%) cow milk to 11.2 mg/100 g in full-fat (3%) cow milk. Walstra and Jenness (1984) reported that milk fat contained 3000 mg of cholesterol per kilogram of lipid and other plant sterols i.e. campesterol, stigmasterol and β-sitosterol were 20, 5 and 10 mg/kg of lipid, respectively. As expected, a higher level of phytosterols was observed in the fortified milk due to the addition of phytosterols to milk in the form of emulsion. Owing to the increase in the phytosterol level among the sterol components, cholesterol percentage in the fortified milk decreased slightly. The amount of cholesterol and total phytosterol of the fortified milk ranged from 8.99 to 9.58 mg/100 g and from 408.49 to 415.5 mg/100 g, respectively, with a mean values of 9.33 and 410.84 mg/100 g, respectively. Hence, one serving (240 g) of

Table 4 Levels of sterols (mg/100 g) in control milk and phytosterol-fortified milk

Sterols (mg/100 g)	Control milk		Phytosterol-enriched milk	
	Range	Mean ± s.d.	Range	Mean ± s.d.
Cholesterol	9.68–10.35	10.01 ± 0.33	8.99–9.58	9.33 ± 0.137
Stimasterol+campesterol	0.176–0.187	0.183 ± 0.04	210.40–214.66	212.06 ± 2.28
β-sitosterol	0.115–0.119	0.118 ± 0.01	197.38–200.84	198.78 ± 1.82
Total phytosterol	0.294–0.306	0.300 ± 0.05	408.49–415.5	410.84 ± 4.03

s.d. = standard deviation.

the fortified milk provides about 49% of the recommended phytosterol level of 2 g/day; two servings of the fortified milk almost the entire requirement.

Effect of storage on sensory and physicochemical properties of phytosterol-enriched milk

The effect of storage on sensory scores of control and phytosterol-fortified milk samples is shown in Table 5. No significant difference was noted for colour between the phytosterol-enriched milk and the control milk over storage. In addition, no significant difference was noted in consistency or overall acceptability between the phytosterol-enriched milk and the control milk at each storage date, but consistency and overall acceptability scores significantly decreased over storage. The flavour score of phytosterol-enriched milk was significantly lower than that of the control milk after 7 days of storage, but flavour scores of both milk samples significantly decreased ($P < 0.05$) over storage. From the results, it can be concluded that the sensory quality of the phytosterol-fortified milk sample was comparable with that of the control milk and was sensorily acceptable up to 7 days of storage.

From the results (Table 5), it can be seen that pH decreased with increasing storage period. A significant difference in mean scores for pH was observed after the seventh day of storage in both control and phytosterol-fortified samples. The results of the present study are in agreement with the work of Zahar *et al.* (1996), who have shown that at 7°C, commercially pasteurised milk samples showed only a slight change in pH after 7 days of storage. In general, when milk is properly pasteurised, packed and stored at 4°C, the growth of microorganisms is low and hence any decrease in pH is expected to be low. Titratable acidity increased with increasing storage period. However, a significant increase was observed after the seventh day of storage in both control and fortified milk samples. The fortified milk had slightly higher acidity than the control sample throughout storage, which, however, did not differ significantly from that of the control sample.

It can be seen from Table 5 that the fortified milk had a higher viscosity throughout the storage period that differed significantly from the control sample. Viscosity of both control and fortified samples increased significantly with the increasing storage period. Regardless of the differences in instrumental viscosity measurements, they were not visibly obvious. Increase in the viscosity of systems indeed is a consequence of the slight decrease in system pH, causing interactions amongst milk proteins (Dzwolak and Ziajka, 1997) and consequently amongst emulsion particles. The MDA levels (mmol/kg) of the fortified milk were slightly higher than those of the control sample throughout the storage period. However, significant differences were not observed between the control and fortified samples on the same day of storage. The sensory evaluation of the product also did not show any oxidative off-flavours during its storage. The oxidative stability of phytosterols in phytosterol-enriched dairy products was analysed by Soupas *et al.* (2006). They evaluated the level of phytosterol oxidation products in phytosterol-enriched milk powder (7% phytosterols) and heat-treated skim milk (0.4% free phytosterols, 0.5% phytosterol esters, 0.5% phytostanol esters) during processing and long-term storage. Non-fat milk enriched with free or esterified phytosterols or with phytostanol esters contained low levels of phytosterol or stanol oxides. No significant changes in the amounts of sitosterol or stanol oxides were detected during 6 months of storage at room temperature or at 4°C. In general, all these phytosterol-enriched dairy products demonstrated stability regardless of the heat treatments used in their processing and long-term storage.

The emulsion stability of the fortified milk was determined by comparison of the fat content of the top and bottom layers of milk samples. There was no significant difference in the fat content between the top and bottom layers of the fortified milk samples during storage, indicating that the samples were properly homogenised and were physically stable. The fat content of the bottom layer was observed to be slightly, but significantly, lower after the seventh day of storage (Table 5).

The phytosterol content of the fortified milk was evaluated after 7 days of storage in three replicates. The mean content of phytosterols of the fortified milk at zero and seventh day of storage was 410.8 and 409.9 mg/100 g, respectively. However, no significant decrease in the level of phytosterols was observed after 7 days of storage. Phytosterol stability

Table 5. Sensory and physicochemical properties of phytosterol-enriched milk during storage (4°C)

Attributes	Sample type	Storage period (days)			
		0	2	4	7
Colour	CM	8.03 ± 0.06^{a1}	8.00 ± 0.09^{a1}	7.96 ± 0.07^{a1}	7.89 ± 0.04^{a1}
	PEM	7.99 ± 0.10^{a1}	7.94 ± 0.04^{a1}	7.92 ± 0.1^{a1}	7.87 ± 0.06^{a1}
Consistency	CM	8.02 ± 0.07^{a1}	7.94 ± 0.09^{a1}	7.87 ± 0.13^{ab1}	7.81 ± 0.02^{b1}
	PEM	8.01 ± 0.08^{a1}	7.94 ± 0.09^{a1}	7.92 ± 0.14^{a1}	7.81 ± 0.02^{b1}
Flavour	CM	7.93 ± 0.09^{a1}	7.72 ± 0.24^{b1}	7.29 ± 0.15^{c1}	7.22 ± 0.26^{c1}
	PEM	7.35 ± 0.17^{a1}	7.27 ± 0.13^{a1}	7.07 ± 0.16^{b1}	6.74 ± 0.12^{c2}
Overall acceptability	CM	7.99 ± 0.01^{a1}	7.87 ± 0.12^{b1}	7.51 ± 0.13^{c1}	7.36 ± 0.17^{d1}
	PEM	7.59 ± 0.11^{a1}	7.48 ± 0.12^{a1}	7.27 ± 0.09^{b1}	7.02 ± 0.05^{c1}
pH	CM	6.67 ± 0.01^{a1}	6.66 ± 0.01^{a1}	6.65 ± 0.01^{a1}	6.62 ± 0.01^{b1}
	PEM	6.57 ± 0.01^{a2}	6.57 ± 0.01^{a2}	6.56 ± 0.012^{a2}	6.54 ± 0.01^{b2}
Acidity (% of lactic acid)	CM	0.15 ± 0.004^{a1}	0.16 ± 0.008^{ab1}	0.16 ± 0.008^{ab1}	0.16 ± 0.005^{b1}
	PEM	0.17 ± 0.005^{a1}	0.17 ± 0.005^{a1}	0.18 ± 0.010^{a1}	0.19 ± 0.009^{b1}
Viscosity (cP)	CM	1.59 ± 0.03^{a1}	1.72 ± 0.04^{b1}	1.77 ± 0.03^{b1}	1.77 ± 0.03^{b1}
	PEM	1.89 ± 0.07^{a2}	2.06 ± 0.11^{b2}	2.17 ± 0.07^{c2}	2.15 ± 0.06^{c2}
TBA (mmol MDA/kg)	CM	0.008 ± 0.002^{a1}	0.011 ± 0.002^{ab1}	0.015 ± 0.003^{b1}	0.015 ± 0.003^{b1}
	PEM	0.013 ± 0.003^{a1}	0.016 ± 0.003^{ab1}	0.019 ± 0.003^{b1}	0.020 ± 0.003^{b1}
Emulsion stability of PEM	Fat% in UL	4.03 ± 0.068^{a1}	4.03 ± 0.096^{a1}	4.05 ± 0.112^{a1}	4.09 ± 0.151^{a1}
	Fat% in BL	4.03 ± 0.068^{a1}	4.03 ± 0.061^{a1}	4.01 ± 0.054^{a1}	3.93 ± 0.062^{b1}

Data represent mean ± s.d. Mean values in each row with different superscripts (a, b, c, d) (by *post hoc* analysis) and in each column for each attribute with different superscripts (1, 2) are significantly different ($P < 0.05$) from each other. CM = control milk; PEM = phytosterol-enriched milk; cP = centipoises; TBA = thiobarbituric acid; MDA = malondialdehyde; UL = upper layer; BL = bottom layer; s.d. = standard deviation.

and antioxidant parameters of phytosterol-enriched functional beverages (milk-based fruit juice, fruit juice and milk beverage) were assessed during 6 months of storage at 4, 24 and 37°C (González-Larena *et al.*, 2012), and the results showed that the studied storage time and temperature did not affect the phytosterol content in the samples. Hence, milk-based beverages are a suitable matrix for the preparation of functional beverages enriched with phytosterols, since they offer the best phytosterol stability during the shelf life period.

Conclusions

Phytosterol is reported to be a valuable nutraceutical substance. Since its level in the diet is low and milk is a food that is regularly used in the diet, fortification of milk with phytosterol could be used to increase dietary phytosterol. Phytosterol being a high melting substance with a poor solubility in milk, its addition was achieved through an o/w emulsion. The fortified milk contained about 410.84 mg of phytosterols in 100 g of the product. The fortified milk compared well with the control milk in respect of sensory and physicochemical properties. The fortified product can be kept for up to 7 days at the refrigerator temperature without any adverse impact on milk quality. Two servings of the fortified milk could provide almost the entire daily requirement of phytosterols.

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